CHAPTER 15. PREDICTING THE FIELD PREY RANGE OF AN INTRODUCED PREDATOR, *RODOLIA CARDINALIS* MULSANT, IN THE GALÁPAGOS

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BACKGROUND

This chapter describes and discusses the procedures used to evaluate the potential threats of a predator, *Rodolia cardinalis* Mulsant, to the conservation of the insect fauna of the Galápagos Islands, a UNESCO world heritage site and biosphere reserve. Due to their late discovery and settlement by humans, the Galápagos Islands are the least altered of any oceanic archipelago (Tye *et al*., 2002). However, Galápagos species are increasingly at risk because of increased human migration to the islands and the associated rise in alien species introductions (Snell *et al*., 2002 a,b). To date, more than 450 species of introduced insects have been recorded as established in the archipelago (Causton *et al*., unpub.). The liberation of *R. cardinalis* in 2002 to mitigate damage to native plants from the invasive scale *Icerya purchasi* Maskell marked the first recorded intentional introduction of an insect into the Galápagos. The risk of introducing a species that might turn out to be a hindrance rather than a help to ecosystem conservation provoked much debate among scientists in the Galápagos. The costs and benefits of introducing *R. cardinalis* were analyzed carefully following the presentation of a risk assessment (Causton, 2001, 2003) that included the feeding range studies that are discussed here.

TARGET PEST: *ICERYA PURCHASI*—A THREAT TO ENDANGERED FLORA

Icerya purchasi (Homoptera: Margarodidae) is a cosmopolitan and polyphagous pest that feeds on at least 200 plant species from many families. The damage to its hosts includes stunting, branch deformation, premature abscission of fruits and leaves, dieback, and even death of the entire plant. Commonly known as the cottony cushion scale, *I. purchasi* is native to Australia but has invaded over 80 countries, primarily through movement of plants or fruit. It is best adapted to tropical and semi-tropical regions (Hale, 1970).

Since it was introduced to the Galápagos in 1982 (on incoming ornamental plants), *I. purchasi* has colonized 15 islands in the archipelago. The spread of this scale insect has been attributed to human activity and dispersal by wind currents (Roque-Albelo and Causton, 1999). Damage by this sap feeding insect was first noticed in 1996, a particularly dry year. Since then, 62 native or endemic species have been recorded as host plants of *I. purchasi*. Sixteen of these species are listed as threatened in the IUCN (International Union for the Conservation of Nature) Red List of Threatened Species, of which six are classified as Endangered or Critically Endangered (Causton, 2001, 2003). Furthermore, the scale's debilitating effect on some plant species, especially those that are already threatened, appears to indirectly affect endemic Lepidoptera that rely exclusively on these species as food sources (Roque-Albelo, 2003).

In 1996, the Charles Darwin Foundation (CDF) and the Galápagos National Park Service (GNPS) identified *I. purchasi* as an invasive species whose impacts required immediate mitigation. Chemical control was not a possible option because of the wide distribution of this pest and because of the impacts pesticides would have on native invertebrates. At the request of the GNPS, the CDF formed a technical advisory committee to evaluate the possibility of employing biological control for the first time on the Galápagos Islands (Causton *et al*., 2004). The committee concluded that studies should be carried out by entomologists at the Charles Darwin Research Station (CDRS), the operative arm of the CDF, to determine (1) whether the detrimental impact of *I. purchasi* on the native flora and fauna was sufficient to merit the introduction of a biological control agent and (2) what risks to the Galápagos biota might result from introducing a natural enemy of *I. purchasi*. The coccinellid beetle *R. cardinalis* was selected as the most suitable biological control agent because of its success in controlling *I. purchasi* in many parts of the world.

RODOLIA CARDINALIS: THE SOLUTION—BUT IS IT SAFE?

Rodolia cardinalis, otherwise known as the vedalia beetle, is believed to be native to Australia (Prasad, 1989). After the successful use of this beetle to control *I. purchasi* on citrus in California in the 1880s, *R. cardinalis* was introduced into over 60 countries. It has successfully established on various continents and islands (Bennett *et al*., 1985; Caltagirone and Doutt, 1989). Because most releases of *R. cardinalis* took place before host testing protocols had been developed, and because of a general absence of post-introduction monitoring, relatively little was known about its feeding range before we initiated our studies.

Many authors have suggested that the range of prey attacked by *R. cardinalis* is narrow and limited to Margarodidae (fluted scales and ground pearls), yet on reviewing the literature and the labels on museum specimens, we found that there was only limited evidence of stenophagy (Causton *et al.,* 2004). This was principally because few autoecological studies had been carried out on this biological control agent. Although some laboratory studies had tested the response of *R. cardinalis* to a few alternate prey such as aphids and mealybugs (Balachowsky, 1932; Kuwana, 1922), these trials did not reveal much about *R. cardinalis*' feeding range. This was because only some of the stages of the predator were tested and crucial information was not included in the description of the methods such as the number of individuals tested and whether they had prior feeding experience, what kind of test arena was used, and whether no-choice or choice tests were used.

Most records of development or feeding by *R. cardinalis* are limited to prey in several genera of Margarodidae, suggesting specialization on this family of scale insects. However, we also found some unconfirmed prey records of *R. cardinalis* feeding on other families of Homoptera, including a dactylopid in its native range of Australia (Frogatt, 1902) and aphids, mealybugs, and armored scales in other parts of the world (R. Booth, pers. comm., 1998; Muma, 1953-54, 1955 as cited by Hodek, 1996; Thompson and Simmonds, 1965). Even though evidence was not available to substantiate these records, we had to assume that *R. cardinalis* might present a risk to these groups. Intraguild predation occurring between *R. cardinalis* and other scale insect predators was also a possibility.

As a result of this preliminary research, the Galápagos advisory committee concluded that there were insufficient data available to fully demonstrate that *R. cardinalis* would not threaten any Galápagos species. At the request of the committee, entomologists at CDRS carried out an assessment of the risks associated with the introduction of *R. cardinalis* that included feeding range tests with potential non-target species.

TESTING LOCATION

Tests were carried out at the CDRS in the Galápagos Islands following a cost-benefit analysis of the economical and logistical advantages of conducting tests "in situ" compared with contracting an organization outside the Galápagos Islands to do the work. Costs were reduced considerably by carrying out the tests in the Galápagos even though it meant building an insect containment facility for this purpose. Not only was it cheaper, but we were also able to test a wider range of species by avoiding the need to ship non-target Galápagos species to another testing location. Tests were carried out from 1999-2000.

DEVELOPMENT OF A LIST OF TEST SPECIES

Step 1: Selection of Criteria for Identifying Potential Non-target Species

To establish the list of non-target species that needed to be tested, we first had to set criteria to define which Galápagos species were most likely to be harmed by the introduction of *R. cardinalis* (Figure 1). To do this, literature on the ecology of *R. cardinalis* and other coccinellid species was reviewed, in particular literature pertaining to foraging behavior, habitat and feeding range. This information provided a preliminary estimate of which families might be used as prey, what characteristics of a prey species might stimulate foraging, and which other species might be directly and indirectly affected. Another important source of information was literature that referred to methods for conducting feeding range tests on predators and parasitoids (e.g., Sands, 1998; Kuhlmann *et al*., 1998; Barratt *et al*., 1999; Keller, 1999; Kirk and Thistlewood, 1999; Sands and Van Driesche, 2000; Lopez and Kairo, 2003). However, because only a handful of entomophagous species have been tested, we also reviewed the literature available for testing weed biological control agents (e.g., Wapshere, 1974; Harley and Forno, 1992).

The following criteria were chosen for selecting species for inclusion in the feeding range tests (see Table 1):

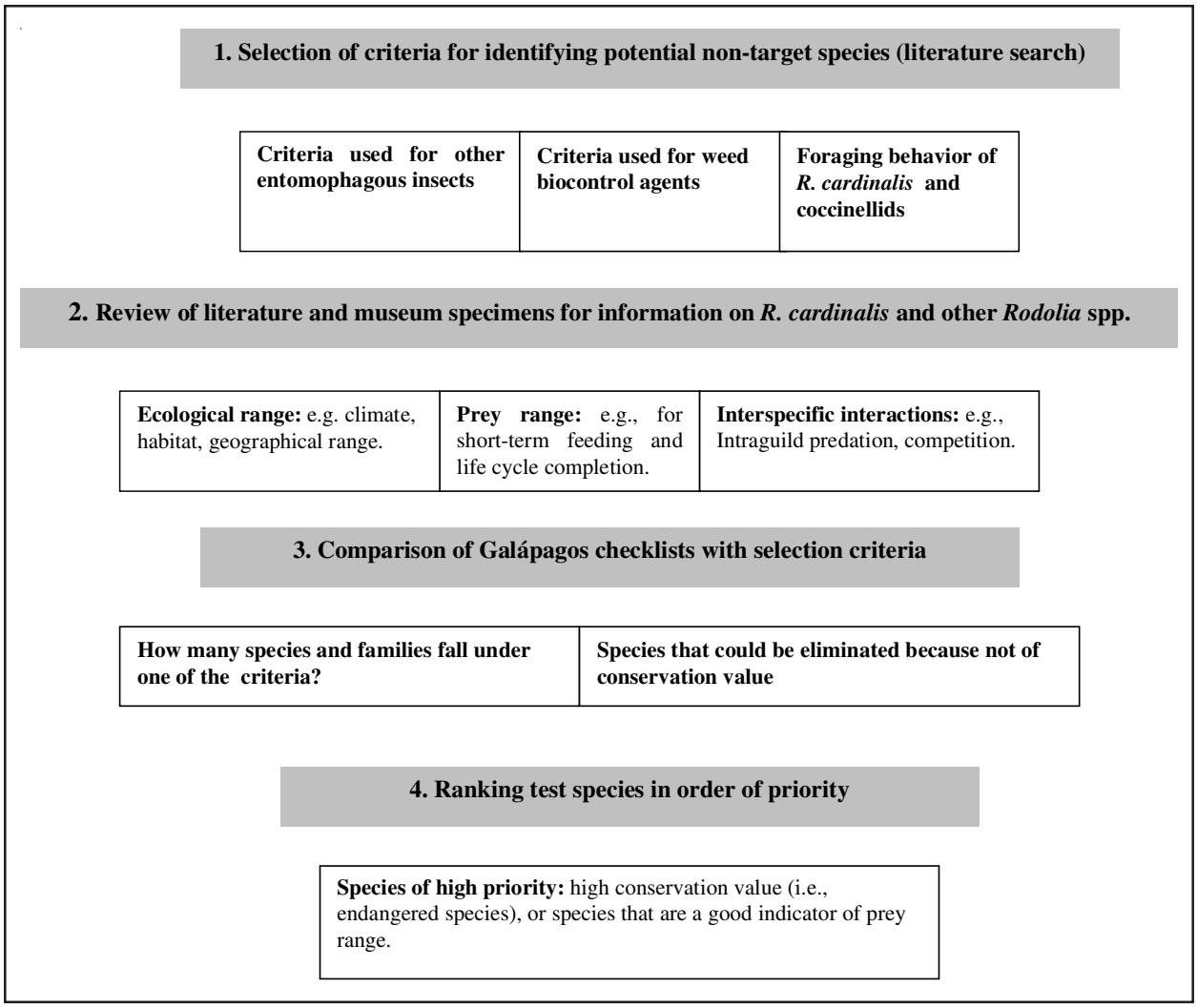

Figure 1. Summary of important considerations for developing a list of test species.

1. **Species closely related to *I. purchasi* or the Margarodidae** Centrifugal testing (Wapshere, 1974), widely used for weed biological control agents, assumes that the closer the species is taxonomically to the target pest, the more likely it is to be attacked.
2. **Species previously reported as prey for any *Rodolia* species** Because coccinellids that prey on scales are known to exhibit restricted feeding ranges (Dixon, 2000), the feeding habits of congenerics were also considered to be a useful indicator of the potential feeding range of *R. cardinalis*.
3. **Species morphologically or physiologically similar to *I. purchasi*** Olfactory and visual cues such as wax filaments produced by scale insects are often necessary to prompt coccinellid foraging and oviposition, (Merlin *et al*., 1996; Dixon, 2000). We assumed that such prey characteristics would influence prey selection by *R. cardinalis*.
4. **Species that live in close proximity to prey of *R. cardinalis*** Species of insects, in particular Homoptera or endangered insects, were considered to be at risk if they occupied niches close to *I. purchasi*. Furthermore, natural enemies that fed either on the pest *I. purchasi* or

Table 1. Groups of Galápagos species potentially affected by *Rodolia cardinalis.*

Selection criteria (relative to target pest)	Nature of impact	Potential prey based on the literature	Groups (number of species present in Galápagos)
Same family	Predation	Margarodidae	*Margarodes similis*
Closely related families	Predation	All Coccoidea	Ortheziidae (1), Eriococcidae (2), Pseudococcidae (7), Diaspididae (3)
Other Homoptera reported as *Rodolia* prey	Predation	Aphididae, Aleyrodidae	Aphididae (3)
Species morphologically similar to *I. purchasi*	Predation	Scale insects with waxy covering	Ortheziidae, Eriococcidae, Pseudococcidae
Unrelated species in close proximity to *R. cardinalis* prey	Competition and predation	Neuroptera, Diptera (Cecidomyiidae, Syrphidae), Hymenoptera, Coccinellidae	Chrysopidae (1), Coccinellidae (10)
Species of conservation value	Toxic reactions produced by feeding	Insectivorous vertebrates	Finches (13), mocking birds (4), warbler (1), lizards (1)

other taxa identified as potential prey of *R. cardinalis* were also considered to be at risk due to competition or intraguild predation. A higher probability of encounter was likely if natural enemies were very common.

5. **Invertebrates of conservation value that might interact with *R. cardinalis***

Step 2: Review of Literature and Museum Specimens for *Rodolia* Species

Sources of information Field studies of *R. cardinalis* in its native range and in countries where it has been introduced, although valuable, were not financially possible. Our knowledge of its feeding range came from the literature and information supplied by museum curators, coccinellid specialists, and biological control practitioners. Databases and search engines on the Internet were also reviewed. Particularly useful sources were Scalenet, CAB Abstracts, and Biological Abstracts. Unfortunately, many of the museum records that we found were not substantiated by published information to confirm whether *Rodolia* species actively fed on the prey listed or were able to complete development on it. We questioned the accuracy of some literature prey records because Hodek (1996) in his review of coccinellids found that adult behavior has often been misinterpreted. He pointed out that finding an adult coccinellid on top of a scale insect is not necessarily an indication that it is feeding on this species. The honeydew of scale insects is often used for short-term survival by coccidophagous insects when their prey is not available. Some host records may reflect insects found feeding on honeydew or merely resting on a branch that happened to have a scale infestation. We decided, however, that the fact that a

species or taxa had been reported as prey meant that it should be considered as a potential non-target species.

Information was also sought on the ecological and geographical range of *R. cardinalis* to determine the likelihood of overlap with potential non-target species in the Galápagos. In addition to this, climate in the Galápagos was compared with that in the beetle's native range using the program Climex (Skarratt *et al*., 1995). Contrary to our predictions, we were unable to find any climatic matches. At the time, we only had 10 years of rainfall data from one Galápagos island available to us (which included an El Niño event) and because the precipitation data used were highly variable, our data were probably not representative of climate in the Galápagos.

Prey records More than half of the 73 existing prey records that we found for *Rodolia* species were simply observations taken from museum labels or other unsubstantiated notes. Feeding range studies have only been carried out for three *Rodolia* species (*Rodolia fumida* Mulsant, *Rodolia iceryae* Janson, and, *Rodolia limbata* Blackburn) that have been used as biological control agents. These tests found that larval development was only possible on margarodids, and in one case only on *Icerya* species (Rasheed *et al.,* 1986; Kairo and Murphy, 1995; Brancatini, unpub.). Except for one unconfirmed record of feeding on mites, our review indicated that *Rodolia* species are restricted to feeding on Homoptera, with 13 out of 21 *Rodolia* species feeding only on margarodids. The remaining species fed on margarodids but were also recorded as preying on other scale insects from the superfamily Coccoidea (in families such as coccids, dactyliiopids, diaspidids, ortheziids, and pseudococcids), in addition to whiteflies and aphids.

For *R. cardinalis* specifically, we found 20 prey records, and this information indicated that the vedalia beetle's prey range was almost entirely restricted to the Coccoidea (Margarodidae, Pseudococcidae, Diaspididae and Dactyliiopidae), with the exception of two unconfirmed reports of feeding on aphids. We found that 12 of the prey records were for margarodids in the genera *Auloicerya, Crypticerya*, *Drosicha, Gueriniella, Icerya, Monophlebus*, *Monophlebulus,* and *Palaeococcus* (Koebel, 1893 cited in Balachowsky, 1932; Kuwana, 1922; Balachowsky, 1932; Anon, 1939 cited in Kairo and Murphy, 1995; Moutia and Mamot, 1946; Bartlett, 1978; Gery, 1991; Ragab, 1995; Mendel *et al*., 1998; V. Brancatini, pers. comm., 2002, 2003). Prey records for *R. cardinalis* also included two genera of mealybugs (Pseudococcidae) – *Maconellicoccus* and *Rastrococcus*; two genera of armored scales (Diaspididae) – *Aspidiotus* and *Selanaspidus*; one dactyliopiid – *Dactylopius*; and one aphid – *Aphis* (Frogatt, 1902; Muma, 1953-54, 1955 as cited by Hodek, 1996; Thompson and Simmonds, 1965; R. Booth, pers. comm., 1998). Prey recorded in *R. cardinalis'* native range were in the genera *Icerya, Monophlebus*, *Monophlebulus,* and *Dactylopius*. *Rodolia cardinalis* has been reported to complete its lifecycle on three genera of Margarodidae (several *Icerya* species, *Palaeococcus* and *Gueriniella*), although it appears that in genera other than *Icerya* life cycle completion is only possible if egg masses are eaten (Balachowsky, 1932; Mendel and Blumberg, 1991). Adults can survive for long periods (up to three months) eating pollen and nectar in the laboratory (V. Brancatini, pers. comm., 1999).

Ecological range *Rodolia cardinalis* is adapted to a wide range of climatic regimes (Bodenheimer, 1951). Biological control with this agent has succeeded in countries with temperate, tropical, or desert climates, suggesting that it would adapt to most parts of the Galápagos if food were available.

Interspecific interactions In the laboratory, larvae of *R. cardinalis* have been observed to kill and or displace larvae of *R. iceryae*, even when target prey were available (Mendel and Blumberg, 1991). Predation may have been involved in the displacement by *R. cardinalis* of congeneric species (*Rodolia koebelei* Oliff and *Rodolia amabilis* Gorham) that fed on *I. purchasi* in California and India (Subramanian, 1953; Bartlett, 1978).

In general, the prey range of *R. cardinalis* and other *Rodolia* species appears to be restricted to Homoptera, specifically scale insects, whiteflies, and aphids. Although one record of feeding on mites was found, mites were not placed on the test list because this record seemed highly doubtful given the known feeding range of the genus *Rodolia*. Other species that might be eaten or displaced by *R. cardinalis* were the natural enemies of potential prey. Because of *R. cardinalis*' tolerance to a wide range of habitats, we concluded that species on the test list might be at risk in any above-ground habitat in Galápagos.

STEP 3: COMPARISON OF GALÁPAGOS CHECKLISTS WITH SELECTION CRITERIA

Checklists for Galápagos Homptera, especially Coccoidea, were found to be incomplete with virtually nothing recorded about species distribution, their host plants, or population status. Consequently, field surveys were carried out in 1999 and 2000 to collect needed information. The discovery of at least four species new to science confirmed our suspicions about the deficiencies of the list. New test species were added to the list even after feeding range experiments had started, and it is likely that the list will grow as new areas in the archipelago are surveyed. A database of these species was compiled.

A list was compiled of all Galápagos species that might serve as prey or otherwise be harmed by *R. cardinalis* (see Table 1). Information was sought on the status of each of these species (e.g., endangered, endemic, native or introduced), their distribution, habitats, abundance, host ranges, and their natural enemies. Following this, we used a process of elimination to exclude any species that were introduced (only native and endemic species were considered of conservation value) or were unlikely to come into contact with *R. cardinalis*, such as gall makers and subterranean species. Based on these considerations, several families were dropped from the test list, including soft scales (Coccidae) and whiteflies (Aleyrodidae).

Ultimately, species from five families of Coccoidea (14 species) and the family Aphididae (3 species) were considered potential non-target prey of conservation value (Table 1). Although host records suggest that *R. cardinalis* is specialized to feed on scale insects, we included aphids in the test list because several records of aphids as prey were found in the literature. We also included three species of Coccoidea that were considered unlikely to be prey because (1) they probably live underground (*Margarodes similis* Morrison and *Pseudococcus insularis* Morrison) or (2) were introduced species (*Paracoccus solani* Ezzat and McConnell). Field studies on *M. similis* confirmed that it lives underground, but this species was retained in the test list because of its taxonomic closeness to the target pest.

Very little is known about the prey ranges of natural enemies of Galápagos Homoptera. A literature search determined that coccinellids, syrphids (Diptera), Neuroptera, and some Lepidoptera are predators of scale insects and aphids in other parts of the world. Galápagos checklists were reviewed and compared with these species, and a list of potential non-target species

was compiled. This list was supplemented by field surveys. In addition, *I. purchasi* populations were monitored for natural enemies for three years.

Only two generalist insect species were found preying on *I. purchasi*: the possibly endemic neuropteran *Ceraeochrysa cincta* (Schneider) and larvae of the moth *Pyroderces rileyi* Walsingham (Cosmopterigidae). The latter species is a new record for the Galápagos, discovered while we were running the feeding tests. It is thought to be an introduced species (Landry, 2001). We do not know for sure whether it fed on detritus (its preferred dietary preference) or was using *I. purchasi* for food. Laboratory studies confirmed our field observations that none of the ten species of Galápagos coccinellids use *I. purchasi* as prey, although one species – *Cycloneda sanguinea* L. – was observed feeding on the honeydew of *I. purchasi* and might interact with *R. cardinalis*. However, encounter rates between *R. cardinalis* and the other species of coccinellids were thought to be fairly high, as all the species are suspected to be coccidophagous or aphidophagous and could occupy habitats that were close to the target prey of *R. cardinalis*. Very little is known about other natural enemies associated with Galápagos Homoptera. During our field surveys we did not collect any native parasitoids or find any predators associated with native Coccoidea or aphids. However, our field trials were limited. Cecidomyiids were collected from two pseudococcids (*P. solani* and *Pseudococcus* n. sp. #6) during the feeding range tests, but it is not yet known if these flies were predators or scavengers.

Rodolia cardinalis might use nectar and pollen as a temporary, alternative food source when prey are scarce and might therefore interact with native pollinating insects in the Galápagos. However, we did not consider this group to be at risk because most insect pollinators in the Galápagos do not specialize on particular plant groups, and thus would not directly compete with *R. cardinalis* for resources. Furthermore, a high proportion of flowering plants do not require insect pollination (McMullen, 1993).

Based on our analysis of the check lists and the feeding behavior of *R. cardinalis* and other *Rodolia* species, we did not consider it necessary to include any additional invertebrate species of conservation value. However, because some toxicity experiments have demonstrated that at least one species of coccinellid (*Coccinella septempunctata* L.) is toxic to vertebrates (Marples *et al.*, 1989), ornithologists were concerned about the potential effect on insectivorous birds and lizards. Accordingly, some such species were included in the test list. Those experiments are discussed elsewhere (Causton, 2003; Lincango and Causton, unpub.).

STEP 4: RANKING TEST SPECIES IN ORDER OF PRIORITY

Because of limited funding and the high costs associated with collecting from other islands in the archipelago, we considered it necessary to identify which of the potential non-target species were most important to test according to their conservation value or importance as an indicator of the prey range of *R. cardinalis*. Because information on the status and ecology of most of these potentially "at risk" non-target species was non-existent, we used host plant distribution as an indicator of their distribution and abundance. Species of highest priority were the endemic species with a small distribution (i.e., those found on a single island) and specialized feeders with a small host range, especially those that are closely related to *I. purchasi* or feed on rare plant species that are also attacked by *I. purchasi* (Table 1). Species with high scores in-

cluded pseudococcids, eriococcids, and ortheziids. *Margarodes similis* was also considered a priority because of its close relationship to *I. purchasi*.

DEFINING TESTING PROCEDURES

In order to fully assess the risks of introducing *R. cardinalis*, our studies needed to respond to three questions.

- Could *R. cardinalis* complete development on other insect species in the Galápagos?
- Are any *R. cardinalis* stages able to switch between prey and feed temporarily on native insects and if so, what degree of population impact do they have?
- Could intraguild predation occur between *R. cardinalis* and natural enemies of scale insects?

Guidelines for defining test procedures and the methods used to assess the prey range of *R. cardinalis* are summarized in Figures 2 and 3.

Step 1: Locating a Source of *R. cardinalis*

Adult *R. cardinalis* were donated by CSIRO Entomology in Brisbane, Australia, from a colony that had been screened and found free of pathogens or parasitoids. The colony originated from beetles collected near Brisbane, Queensland. Our colony of *R. cardinalis* was maintained in the quarantine facility at CDRS and was fed on field-collected *I. purchasi* and honey.

Step 2: Background Research for Choosing a Test Procedure

Our goal was to use stages of the predator, test species, and environmental conditions that would most accurately predict the field prey range of *R. cardinalis* in the Galápagos. Achieving this goal required information about the ecology and biology of *R. cardinalis*, as expressed in the following questions:

- Does *R. cardinalis* oviposit on its prey or elsewhere;
- Do confined spaces or any other factors stimulate oviposition in the absence of the host;
- Are olfactory, tactile or any other environmental cues needed to prompt oviposition and foraging, such as specific plant chemicals and morphological features;
- At what age is beetle oviposition highest and how long is the oviposition period;
- Are all larval stages mobile;
- What stages of *R. cardinalis* feed on prey that might be valuable native species;
- Which is the most voracious feeding stage;
- Are any stages cannibalistic?
- What stages of prey does *R. cardinalis* feed on;
- Does *R. cardinalis* feed on parasitized prey; and
- Could prior feeding experience influence prey selection?

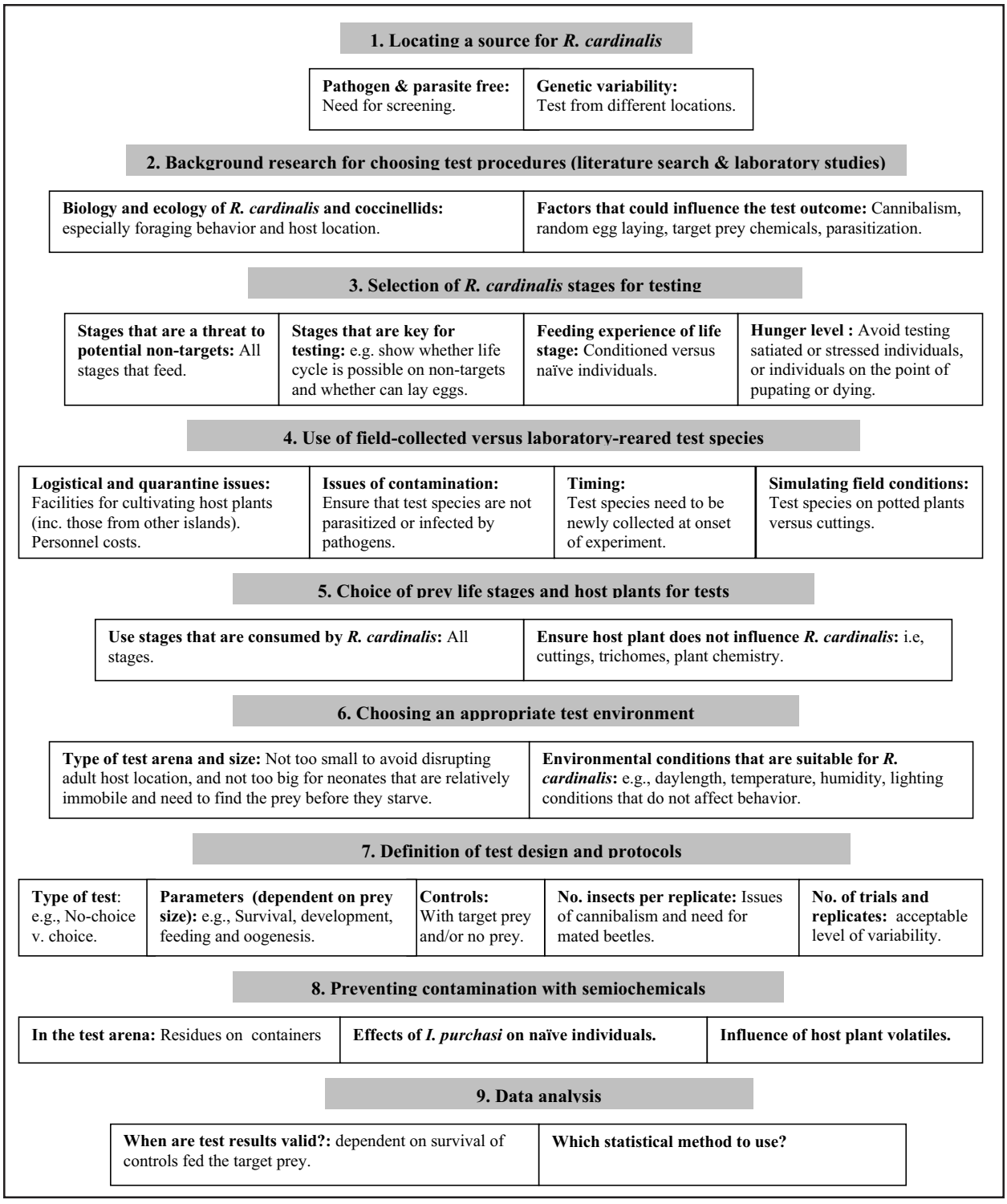

Figure 2. Summary of important considerations for defining test procedures.

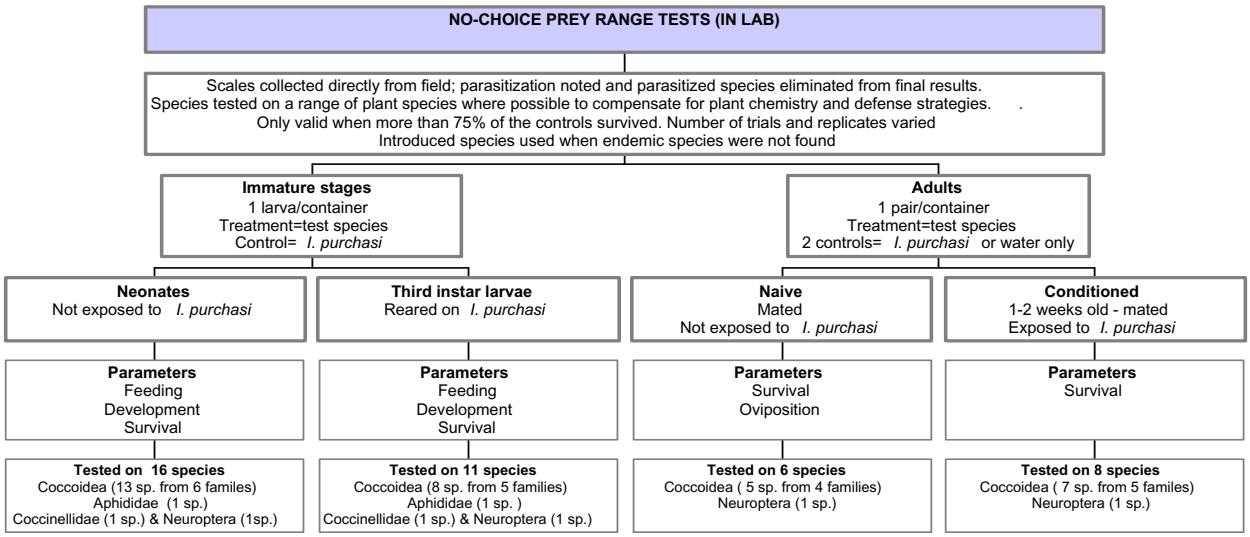

Figure 3. Summary of procedures used for feeding range trials of *Rodolia cardinalis.*

STEP 3: SELECTION OF *R. CARDINALIS* STAGES FOR TESTING

Both adults and larvae of *R. cardinalis* are entomophages and were considered potential threats to non-target species in the Galápagos. Literature and our preliminary studies indicated that *R. cardinalis* lays eggs on or near *I. purchasi* and larvae are initially weak, suggesting that adults define the prey range of recently emerged larvae. Although recently eclosed larvae are only likely to be a threat to a non-target species if the adult has selected it for oviposition, we found that adults that had consumed the target prey (conditioned adults) laid eggs in empty test arenas and that oviposition could not be used as a reliable parameter for testing prey selection. First instar larvae were therefore chosen to determine whether non-target prey could support complete development.

Mature larvae and adults were also selected for testing because our preliminary studies showed that they were voracious feeders and very active, and both of these stages had the potential to encounter other prey species. Prasad (1990) found that adults have a capacity to move over long distances in the field, increasing the probability that they could be found outside the range of its target prey. Although temporary foraging on non-target species is considered acceptable and sometimes necessary for sustaining population numbers of the agent when its target prey is low (e.g., Sands, 1997; Sands and Van Driesche, 2000), in a conservation context such as in the Galápagos, short term feeding by a voracious predator may have considerable impact on non-target species, especially on already threatened endemic species.

Because coccinellids are unable to develop eggs until they have fed on a prey that is nutritionally adequate (Matsuka *et al*., 1982; Frazer, 1988), we also considered naïve adults in tests of the suitability of non-target species to support oogenesis.

Hunger level and condition of life stage When choosing the stage of a predator for prey range testing, it is also important to ensure that individuals are at a point in their life cycle when they

would consume food. For example, it would have done no good to test fourth instar larvae that are on the point of entering the prepupal stage or to test adults that were past peak egg laying, as old adults required less food and died quickly when starved. Consequently, we tested late second and early third instars, which were active and readily consumed prey. Deciding how old adults should be for testing proved to be more complicated. According to Cressman (1930), female beetles eat the most in the first third of their adult lives, following their preoviposition period of 3 to 28 days. However, in preliminary trials, we had observed that the survival rates of adults that had been removed from the target prey varied with age. To ensure that adults were exposed to non-target prey at an age when they would exhibit maximal feeding and to provide a sufficiently long exposure period to the test species, we conducted trials to evaluate the effects of eliminating *I. purchasi* from the diet of *R. cardinalis* adults after 3 days, 1 week, 2 weeks, or 4 weeks after beetle emergence. Ten replicates were tested in each trial, and each replicate consisted of a 9 cm dia. petri dish with a newly emerged female-male pair and two adult female *I. purchasi*. Using an ANOVA followed by a least significant difference LSD means separation process (using the SPSS system in Norusis, 1993), it was determined that beetles removed from a *I. purchasi* diet after 3 days ($P < 0.001$) or one week ($P < 0.05$) lived longer than did beetles that had fed on *I purchasi* for four weeks. Females lived significantly longer ($P < 0.001$, $\overline{X} = 5.7$ days, SD = ± 1.8, n = 38) than males ($\overline{X} = 4.4$ days, SD = ± 2.2, n = 38) when the results were pooled across age classes. Female longevity may have been dependent on reproductive output, with survival in the absence of prey declining in proportion to the number of eggs already laid (see Dixon, 2000). Because we were interested in assessing the prey range of beetles that had sufficient prior feeding experience on the target pest, we decided to test beetles fed on *I. purchasi* for 1 to 2 weeks.

We also asked whether prey selection by the different stages of *R. cardinalis* would be influenced by previous feeding on *I. purchasi*, and if so, might recently emerged larvae and adults that had never been exposed to the target prey behave differently and perhaps eat prey that conditioned adults would reject. To test this hypothesis, naïve, unfed neonate larvae were tested instead of first instar larvae that had already fed on *I. purchasi*. Recently emerged, naïve adults were also tested.

Hunger levels can also influence the outcome of feeding experiments. Satiated individuals often do not respond quickly to prey, while naïve (unfed) individuals may become weak and uninterested in feeding if not tested immediately. In our experiments, conditioned adults were separated from *I. purchasi* and given water but no food for 1-2 days. This was not done when mature larvae were assessed: these were transferred directly to the test arena from containers stocked with *I. purchasi*. Eggs were checked first thing in the morning and regularly throughout the day so that neonates were exposed to a test species soon after emerging. Sluggish individuals were not selected for testing. Naïve adults were kept in plastic containers for a day following their emergence to ensure that they had mated and would be able to lay eggs in the event that they fed on a suitable host.

The rearing conditions of the colony also influenced the state of the life stages used in the trials. An adequate food supply and small number of *R. cardinalis* in each rearing container were important factors in ensuring that beetles were healthy. Crowded containers produced smaller individuals, which, in some coccinellid species (e.g., Booth *et al.*, 1995), reduces fecundity.

In summary, neonates were tested for life cycle completion on a non-target species and to assess conditioning due to prior prey consumption. Mature larvae were used to test their ability to switch between prey species. Naïve adults were used to test their ability to develop and deposit eggs after feeding on non-target species and assess whether or not previous prey contacts influence prey selection. Conditioned adults were used to test adult's ability to switch between prey species. All life stages were tested in separate experiments.

Step 4: Use of Field-collected Versus Laboratory-reared Test Species

At an early stage, we concluded that the advantages of testing field-collected insects far outweighed testing laboratory-reared individuals. Too little was known about the non-target prey and their host plants and how to cultivate the host plant and use them to rear colonies of test species in the laboratory. In addition to this, because some of these species were found only on islands other than the one we were working on, it would have involved rearing the species under quarantine conditions, which was not logistically or economically possible.

The principal disadvantages of using field-collected insects were that the test species needed to be collected just before the experiments were started and did not survive long once they were collected. This limitation coupled with the need for specific *R. cardinalis* stages made conducting experiments difficult. Another disadvantage of using field-collected prey was that, in the event that results were not significant or were invalid, it was difficult to repeat the experiments until new collections could be made. The post-El Niño conditions prevalent at the time of the trials had lowered the numbers of most of these species, making subsequent collections difficult. Nor could we test adults under simulated field conditions by using potted plants in large cages.

There was also the possibility that some field-collected material would be parasitized or contaminated by pathogens that might not be detected until experiments were underway. However, our surveys showed that few endemic or native species had associated parasitoids or pathogens. Throughout our trials, only three prey species were parasitized (14% of the Homoptera tested), two of which were introduced species while the third was of unknown origin. Although *R. cardinalis* has been known to eat parts of *I. purchasi* parasitized by the dipteran *Chryptochaetum iceryae* Williston in times of prey scarcity (Quezada and Debach, 1973), we decided that it was better to eliminate any test species that were parasitized or diseased. This was in part because little was known about the response of *R. cardinalis* to the presence of other parasitoids. As a precautionary measure, test material and any additional material that wasn't used in the trials were reared after the trial to check for parasitoids. Additionally, two prey species were found to be infected by fungi and were excluded from the final analysis.

Step 5: Choice of Prey Life Stages and Host Plants for Tests

Life stages In principle, we wanted to test all life stages of each test species because all stages of *I. purchasi* are consumed by *R. cardinalis*. Early instars of the test species were always included in tests with neonates because neonates' mouthparts may be unable to penetrate the tougher integuments of older stages of some species. In practice, however, the life stages that were tested depended on what was available at the time (see Tables 2 and 3). Test prey were supplemented every three days to ensure that there was a sufficient food supply and plants were fresh.

Table 2. Suitability of potential non-target prey for the development of immature stages of *R. cardinalis.*

Test prey Species [a, b]	Development of *R. cardinalis* larvae					
	Neonates[c]			Third instars[c]		
	Feeding	Development	n	Feeding	Development	n
Ortheziidae (Homoptera)						
Orthezia insignis (I)	—	—	15	—	—	10
Orthezia sp. (?)	—	—	21	Nt	Nt	Nt
Margarodidae (Homoptera)						
Margarodes similis (E) (cysts) •	—	—	88	—	—	26
M. similis (emerged females) •	+	—	94	+	—	3
Pseudococcidae (Homoptera)						
Antonina graminis (N?)	—	—	57	—	—	45
Pseudococcus n. sp. # 2 New sp. •	—	—	20	—	—	14
Pseudococcus n. sp. # 3 New sp. •	—	—	44	—	—	22
Pseudococcus sp. (?)	—	—	26	—	—	17
Paracococcus solani (N?) •	—	—	15	Nt	Nt	Nt
Eriococcidae (Homoptera)						
Eriococcus papillosus (E) •	—	—	69	—	—	15
Coccidae (Homoptera)						
Saissetia coffeae? (I)	—	—	11	Nt	Nt	Nt
Parasaissetia nigra (I)	—	—	20	Nt	Nt	Nt
Diaspididae (Homoptera)						
Selenaspidus articulatus (I)	—	—	20	—	—	31
Aspidiotus excisa (I?)	—	—	15	Nt	Nt	Nt
Aphididae (Homoptera)						
Sitobion sp? (E?) • (all stages except eggs)	—	—	69	—	—	25
Coccinellidae (Coleoptera)						
Pentilia sp. (E?) • (mature larvae, pupae and adults)	—	—	8	—	—	28
Chrysopidae (Neuroptera)						
Ceraeochrysa cincta (E?) • (eggs not tested on third instar larvae)	—	—	26	—	—	24

[a]All stages tested unless indicated;
[b](E) = endemic; (I) = introduced; (N) = native; • = high risk potential prey of conservation value;
[c]— = negative response; + = positive response; Nt = not tested

Food plants Attempts were made to reduce the effects of plant chemistry and plant defenses on the outcome of the tests. Where possible, several food plants were used for test species that used more than one genus as a resource, and plant species that are toxic to insects were avoided (see Step 8). Additionally, we tried to use whole leaves rather than parts of leaves because the chemistry of plants that are cut may be altered and affect prey selection (see Palmer, 1999). We also tried to avoid using species with trichomes and pronounced pubescence that might influence the foraging behavior of the prey, as we had observed that neonates found it hard to walk on some of these species. Furthermore, several authors (e.g., Eisner *et al.*, 1998; Gamarra *et al.*, 1998) have found that coccinellids can be killed or lacerated by trichomes.

Table 3. Survival (number of days) of "conditioned" and "naïve" adult *R. cardinalis* fed on a test prey species compared with individuals given only water (NC).

Test prey species[a]	Survival (days ± SD)[b]							
	Naïve				Conditioned			
	Test	n	NC	n	Test	n	NC	n
Margarodidae (Homoptera)								
Margarodes similis (E) (emerged female)•	10.5 ± 3.8**	10	3.8 ± 1.0	10	5.8 ± 4.3	10	3.1 ± 0.5	10
M. similis (cysts)•	5.5 ± 1.3	10	4.7 ± 1.3	10	2.8 ± 0.3	10	3.4 ± 0.4*	10
	7.8 ± 1.1	11	7.6 ± 2.0	11	Nt		Nt	
Pseudococcidae (Homoptera)								
Paracoccus solani (N?)	6.7± 0.9*	12	5.4 ± 1.0	11	2.0 ± 1.6	17	1.9 ± 0.7	17
	Nt		Nt		3.0 ± 0.7	17	2.9 ± 0.8	17
Pseudococcus sp. #3 New Sp.•	Nt		Nt		3.6 ± 1.2	14	2.8 ± 0.8	13
Pseudococcus sp. #6 New Sp.•	3.9 ± 0.8	8	4.8 ± 1.3	7	2.0 ± 0*	5	1.2 ± 0.4	5
Eriococcidae (Homoptera)								
Eriococcus papillosus (E)•	5.9 ± 1.8	9	4.6 ± 1.4	10	4.2 ± 1.0*	4	2.3 ± 0.6	3
Coccidae (Homoptera)								
Ceroplastes rusci (I)	6.3 ± 1.1	9	6.4 ± 1.7	9	4.1 ± 0.6	7	3.8 ± 0.9	7
	Nt		Nt		4.4 ± 0.5	4	3.9 ± 0.2	4
Diaspididae (Homoptera)								
Aspidiotus excisa (I?)	Nt		Nt		3.1 ± 0.7	13	3.4 ± 0.6	13
Chrysopidae (Neuroptera)								
Ceraeochrysa cincta (E?)•	2.5 ± 1.5	16	NA		3.6 ± 1.3	5	NA	
	Nt		Nt		1.2 ± 0.4	6	NA	

[a](E) = endemic; (N) = native; (I) = introduced; • = potential prey of conservation value

[b]Sample means compared using independent samples t-test for data with equal variance and Mann-Whitney U test in the event of unequal variation. NA = Not applicable, * = significant (P<0.05), ** = highly significant (P<0.001)

STEP 6: CHOOSING AN APPROPRIATE TEST ENVIRONMENT

Type of test arena and size Because neonates are virtually immobile, we used a small test arena to guarantee that the predator would encounter the non-target prey. Eppendorf tubes were found to be too big (mouth = 1 cm dia., 4.2 cm high), but were acceptable when the area was reduced by inserting a plug made from Kimwipes® and leaving a 1 cm long space for the larval movement (Figure 4). This methodology was based on similar experiments with *R. limbata* (V. Brancatini, pers. comm., 1999). One of the problems with using this method was that larvae would sometimes burrow into the plug. Orienting the tubes narrow end down reduced this problem. We did not put any water in the containers because preliminary trials showed that even the smallest drop drowned larvae.

Late instar larvae and adults were tested in 9 cm dia. petri dishes (Figure 5). Studies on other entomophagous coccinellids suggests that proximity to the prey stimulates foraging (Samways and Wilson, 1988; Dixon, 2000), and we concluded that the use of a small arena should not disrupt prey location cues. Previous studies with *R. cardinalis* indicated that it would mate and lay eggs in containers of this size (Matsuka and Watanabe, 1980; Ragab, 1995).

Figure 4. Eppendorph tubes were used to test neonate larvae. This photo shows a positive control using *I. purchasi* and a *R. cardinalis* larva. Photo: Heidi Snell. (UGA1295010)

Figure 5. Eggs of an endemic mealybug tested against *R. cardinalis* adults. Indeterminate numbers were used because of their small size. Photo: Heidi Snell. (UGA1295009)

Environmental conditions All trials were conducted at 24-26 °C, 60% average relative humidity, and 12:12 L:D photoperiod. We found that these were acceptable conditions for *R. cardinalis*. Fluorescent bulbs with high frequency electronic ballasts (1500 hz) were used to avoid promoting irregular insect behavior (A. Cross, pers. comm., 1999).

STEP 7: DEFINITION OF TEST DESIGN AND PROTOCOLS

Type of test – no-choice versus choice We selected no-choice tests because we were primarily interested in seeing if *R. cardinalis* would feed and survive on non-target species rather than in demonstrating differences in predator preference among prey species. Responses in tests of starved larvae or adults to a non-target species (the treatment - T) were compared with the response of individuals offered the target prey (the positive control). Tests thus created an "eat it or die" situation. Although, there was some risk of false positives (feeding on a species that *R. cardinalis* would not normally feed on under field conditions), we felt that there were fewer external factors in this design that might affect prey selection. In choice tests, the presence of semiochemicals from the target prey or another prey can lead the predator to ignore an alternative test prey, inducing a false negative result. Furthermore, use of no-choice tests allowed us to quickly eliminate those species not eliciting feeding from the list of potential prey. This allowed us to screen a larger number of prey species.

Parameters and frequency of measurements To score responses in our no-choice tests, we measured predator survival (number of days alive) to determine if naïve or conditioned adults could feed on non-target prey. For predator larvae, we measured both survival and development (the presence of larval molts). Although molts might suggest feeding, larvae chosen for tests could be close to molting when they were placed in the test arena, and caution should be used in interpreting such events. If feeding was seen, it was recorded, but the number of prey eaten could not be measured because prey were small and numerous, and were continuously emerging from pupae and eggs during the experiments. The number of fecal pellets deposited by adult predators was initially counted but was not used in the analysis because both starved

naïve and starved conditioned beetles produced a small number of feces in some trials. In addition, we recorded the number of eggs deposited by adults.

Notes on the behavior of *R. cardinalis* (e.g., location of beetle in the test arena, degree of mobility, and indications of feeding) were taken at least twice daily, once between 8.00 and 10.00 h and again between 15.00 and 17.00 h. Test prey were examined for signs of predation when the food supply was changed (every three days).

Controls. To provide experimental controls in all trials, response data were collected for larval and adult predators taken from the same rearing batch and exposed to the normal prey or confined with water only. Positive controls (PC) using the target prey were used to confirm that the predators were capable of normal feeding and development. In one of our trials, for example, *R. cardinalis* was observed to be sluggish and control beetles didn't feed on *I. purchasi*. We later discovered that those beetles were infected with a pathogen, and we had to restart the source colony. The use of such positive controls also enabled us to compare larval development rates of controls with those of individuals reared on various test species.

Because we were not able to directly measure feeding, we compared survival time when beetles were exposed to a test species to survival time with water alone. This was especially important for adults, for which – unlike larvae – there were no obvious ways to observe growth as a consequence of food intake. We reasoned that, if feeding was taking place, then beetles would live longer than starved beetles, which acted as negative controls (NC). In retrospect, it would have been useful to have also included such negative controls for larvae.

Only two treatments (T and PC) were used for testing adults against other predators because we were more interested in directly observing the interactions between the two species rather than measuring survival.

Number of insects per replicate We set the number of predator larvae per replicate at one because the immature stages of *R. cardinalis* are cannibalistic. For adults, we used a female-male adult pair in each replicate to ensure that naïve females had mated, even though males did not live as long as females. Mean survival time for adult predators was calculated for each replicate. When only one sex of the predator was available, the same sex was used for all treatments.

For species of prey, the exact number of a test species present in a trial was usually unknown because of the small size of most species, the difficulty in counting them (see Figure 5), and the fact that new prey hatched from eggs during the trials. In most cases, several individuals of different stages of each species were included in tests.

Number of trials and replicates As replication, our goal was to run 15 to 20 replicates per test species per trial and repeat a trial at least twice. Ultimately, the number of prey tested depended on their availability and that of the predator. Across all prey species the number of trials varied from one to seven ($\overline{X}$ = 1.88), and the number of replicates from 3 to 31 ($\overline{X}$ = 12). When the number of replicates in the trial with a given prey was low (< 7) or if the prey species occurred on many host plants, we increased the number of trials. If we knew that a given prey would be difficult to obtain a second time, we increased the number of replicates in a trial. When we only had a small number of a scarce species, trials were run even if the number of replicates was low (< 4). In all cases, we maximized the number of trials and replicates devoted to testing neonates because we considered that this was the most crucial stage to be tested.

Duration of experiments A treatment and its corresponding control(s) (together being one replicate) were run at the same time. However, because it was difficult to have enough predators ready at the same time, replicates were staggered over many days. Trials were terminated 7 days after all the individuals that had been exposed to the test prey species and the control with only water (NC) had died.

STEP 8: PREVENTING CONTAMINATION WITH SEMIOCHEMICALS

In the test arena To reduce the possibility of volatile chemicals from test insects influencing prey selection, each species and its control was placed in a different perspex cage (50 x 50 x 50 cm). Cages with *I. purchasi* were placed at the other end of the room from the treatment cages. (We were unable to keep them in separate rooms due to space constraints.) Petri dishes were recycled because of limited materials and were washed in a biodegradable and odorless detergent with a final rinse in a 1% Clorox bleach solution. The perspex cages were washed in the same manner after each experiment.

Minimizing the effects of* I. purchasi *on naïve individuals To reduce exposure of naïve neonate *R. cardinalis* larvae to chemical volatiles from *I. purchasi*, we isolated mature *R. cardinalis* adults (previously fed on *I. purchasi*) in plastic containers (11 cm dia.) with cotton balls. Isolated adults were fed honey and water, and after three days, eggs in the cotton wool were placed in a clean container for larval emergence. To obtain naïve adults, we isolated two-day old pupae, dipped them in 1% Clorox solution, and placed them in a sterile container for adult emergence. This method may not have been completely effective in eliminating volatiles from *I. purchasi*, but other solvents were not available.

Minimizing the influence of host plants on prey selection Alkaloids are sequestered by the scale *I. purchasi* from several species of Leguminosae, Aceraceae, and Menispermaceae that deter *R. cardinalis* from feeding on the scale or make it less suitable for predator development (Quezada and Debach, 1973; Mendel and Blumberg, 1991; Mendel *et al*., 1992). Before running our trials, we checked the likely Galápagos host plants of non-target prey against a list of plant genera known to produce alkaloids. We also fed *R. cardinalis* on *I. purchasi* reared on as wide a range of host plants as possible to see if there were any plant species that influenced prey selection. To our knowledge, none of the prey species we used fed on plant species with toxic alkaloids.

STEP 9: DATA ANALYSIS—WHEN ARE TESTS RESULTS VALID?

Trials were only considered valid when more than 75% of the controls that fed on *I. purchasi* survived. We did not use any statistical method for analyzing data on larval survival because the prolonged process of feeding on prey and the existence of larval molts made it easy to detect feeding or development. Furthermore, water-only controls (NC) were not used for comparison. For adults, the average survival time was calculated for each treatment. Because the control groups fed on *I. purchasi* were terminated approximately one week after the beetles from the other treatments died, data were not normally distributed. Consequently, a Kruskal-Wallis test was used to detect significant differences in survivorship between treatment and control means. An independent sample t-test analysis was used to determine significant differences between treatments (T) with the test species and the negative controls with no food (NC) if equal vari-

ance was confirmed by the Kruskall-Wallis Test. The Mann-Whitney U test was used in the event of unequal variance. The statistics were calculated with the SPSS system (Norusis, 1993).

TEST RESULTS AND INTERPRETATION

LARVAE

Results were considered valid for 16 species (from nine families) for tests with neonate larvae and for 11 species (from eight families) for tests with late instar larvae (Table 2). Test species included members in three insect orders (Homoptera, Coleoptera and Neuroptera). Larvae of *R. cardinalis* only fed on *M. similis*, a congeneric of the target pest. Only females of *M. similis* that had emerged from their protective waxy cysts were consumed. Neonate larvae lived up to 7 days ($\overline{X}$ =1.7 days, SD = ± 1.5, n = 94) on *M. similis*, but were unable to molt to second instar, suggesting that *M. similis* adults were not suitable for development. On all other prey species, *R. cardinalis* larvae died within 1 to 2 days. Because *M. similis* became unavailable in the field and could not be reared in the laboratory, only three late instar *R. cardinalis* larva were tested on this species. All three larvae completed development to the adult stage, but we were unable to observe whether they were able to develop and reproduce. Mature larvae did not feed on any other prey species offered, although they could live for up to 15 days, which was equal to the time taken for larvae feeding on *I. purchasi* to complete their development.

Although, *R. cardinalis* larvae did not feed on or kill the two predators tested (*C. cincta* and *Pentilia* sp.), on one occasion a mature larva of *R. cardinalis* and the *Pentilia* sp. were found with their jaws locked together. Conversely, larvae of the lacewing were often observed extracting the fluids from dead or dying *R. cardinalis* larvae. In addition, preliminary observations showed that *R. cardinalis* larvae did not approach a *Diomus* species (Coccinellidae) or the lepidopteran *P. rileyi.*

ADULTS

Representatives from two insect orders (Homoptera and Neuroptera) were successfully tested against adults of *R. cardinalis* (Table 3). Adults with prior feeding experience on *I. purchasi* were tested against eight non-target species from six families, and naïve adults were tested against six species from five families. As with the immature stages, we observed that both conditioned and naïve adult *R. cardinalis* beetles fed on females of *M. similis* that had emerged from cysts. Naïve, mated *R. cardinalis* adult pairs given emerged *M. similis* females lived significantly longer ($\overline{X}$ =10.5 days, SD = ± 3.8, n = 10, $P < 0.001$) than starved individuals (treatment NC) ($\overline{X}$ =3.8 days, SD = ± 1.0, n = 10). Moreover, 65% of the beetles survived for more than 13 days, at which stage experiments had to be terminated due to a shortage of *M. similis*. On the other hand, the longevity of beetles previously fed on *I. purchasi* and then exposed to *M. similis* was not significantly different from that of the negative control beetles (NC) fed only water. Adult beetles were unable to break open the hard waxy cysts that typically protect *M. similis* females, and the presence of the cysts in the test arena did not result in beetles living longer than individuals that were starved.

We did not observe naïve or conditioned adults feeding on other species of Coccoidea and did not find any obvious signs of feeding (such as punctured ovisacs and torn scale insects). Beetles rarely settled on test Homoptera and were very active, moving continuously in circles around the dish. Conditioned *R. cardinalis* adults tested against six additional scale insect species lived for an average of 3.1 days (SD = ± 1.3, n = 81) and did not live any longer than the controls (NC) held with water only ($\overline{X}$ = 2.7 days, SD = ± 1.0, n = 79) in 75% of the trials. Beetles tested against a new species of *Pseudoccccus* sp. #6 and *E. papillosus* lived longer than the controls within the same trial ($P < 0.05$). However, only beetles tested against *E. papillosus* lived longer ($\overline{X}$ =4.2 days, SD = ± 1.0, n = 4) than the average for conditioned beetles given only water when the trials were pooled for conditioned beetles tested against Homoptera ($\overline{X}$ =2.8 days, SD = ± 1.0, n = 99). Likewise, naïve *R. cardinalis* adults tested against three out of four species did not live any longer than controls given only water, while adults tested against the pseudococcid *P. solani* lived significantly longer ($\overline{X}$ = 6.7 days, SD = ± 0.9, n = 12, $P < 0.05$) than both their water-fed counterparts and the average for water-fed controls when data were pooled across all trials with naïve adults tested against Homoptera ($\overline{X}$ =5.4 days, SD = ± 1.8, n = 68). Adults were not observed feeding on larvae of the lepidopteran *P. rileyi* or larvae of the lacewing *C. cincta*. In contrast, adults that were weakened by a lack of food were often attacked by this neuropteran. None of the species exposed to naïve beetles were suitable for egg development, including *M. similis*. Egg laying was only observed after individuals had eaten *I. purchasi*.

Excluding the trials conducted on emerged *M. similis*, mean survival time was marginally or significantly higher for both naïve and conditioned adults fed on the test Homoptera compared to those fed only on water in 73% of the trials (n = 15). However, in all trials where Homoptera were tested, the maximum number of days an individual remained alive did not differ markedly between the controls and test species. Because we didn't find any evidence of feeding, we concluded that increased survivorship might have been because adults either fed on honeydew or attempted to feed on the test prey. It is also likely that the presence of Homoptera might have stimulated beetles to forage for longer before giving up. Significant differences in lifespan were noted between the treatments and controls in both tests with naïve and conditioned *R. cardinalis* adults. This suggests that prior feeding experience may not influence host selection. By repeating these trials we would have had a clearer idea of the response of adult *R. cardinalis* to families other than Margarodidae; however, by the time that the results were analyzed, the test species were unavailable.

PROBLEMS ENCOUNTERED WITH TESTING PROCEDURE

A summary of shortcomings and how we dealt with them is shown in Table 4. The principal setbacks encountered during the feeding trials are discussed below.

DIFFICULTY IN LOCATING TEST SPECIES

Our biggest problem was finding the species that we needed to test. Many of the species that were identified as potential non-target prey were found only on islands far from that where the host testing was carried out. Inter-island transport is very expensive in the Galápagos, and this

Table 4. Summary of problems and solutions encountered during feeding tests.

Shortcomings	Our solution	Ideal
Unable to determine prey range of *R. cardinalis* in the field	Literature and museum databases searched extensively. Specialists contacted.	Conduct exploratory surveys in *R. cardinalis'* native range or countries where it has been introduced.
Little known about the foraging behavior of *R. cardinalis* and factors that might influence test results.	Preliminary behavioral studies conducted. Predictions made based on current knowledge of the behaviour of Coccinellidae.	Carry out in-depth behavioral studies.
Checklist of Galápagos species incomplete.	Field surveys conducted. Deductions based on what is known from other parts of the world.	Survey extensively.
Field survey for test species limited by budget.	Ranked potential non-target species according to priority for testing. Tested species that had not been identified as potential non-targets but were from the same families as potential non-targets.	Amplify surveys.
Rearing of test species in laboratory prevented by space, budget, and quarantine constraints.	Collected material directly from field.	Rear high priority test species on plants to obtain colonies free of natural enemies and pathogens.
Difficulties locating the target prey, *I. purchasi*.	Searched far and wide on island for healthy infestations.	Maintain colonies on potted plants in cages.
Difficulties evaluating whether adult *R. cardinalis* fed on test species.	Measured survival (number of days alive) and compared this with controls fed only water.	N/A
Contaminants: insect and plant semiochemicals	Washed test arenas thoroughly and separated the arenas with the target prey from those that contained the test species. Used host plants that are not known to produet alkaloids.	Maintain test species and controls in different rooms. Wash containers with solvents suitable for eliminating volatile chemicals or use new containers. Test species on a range of host plants and without host plant.

precluded us from collecting some of the species reported from the outlying islands. Moreover, because some species had only one known collecting record (e.g. the Ortheziidae species), we could not predict the best time to collect them, so trips often failed to locate desired insects. Extended dry periods following an El Niño event caused many plants to dry out, which fur-

ther exacerbated the problem, especially for testing against *R. cardinalis* adults. It also limited the range of host plants on which each non-target species could be tested and prevented us from repeating some tests. Moreover, for some species, specimen labels were very vague about the host plant (e.g., "under yellow plumed plant"!), making it difficult to locate the species.

In order to increase the number of species tested against *R. cardinalis*, we opted for a find-and-test approach, testing any likely species that we came across, even if they were introduced species. This let us increase the range of species tested against *R. cardinalis* and better determine its feeding range. Given the circumstances, we considered that even just testing species from the same family as a potential non-target species was valuable.

Keys were not available, so that once a species was located, its identification had to be confirmed by sending the specimen off to a scale insect taxonomist. Because this was time consuming and because we often needed to test the species immediately, we often tested a species before we knew what it was.

TARGET PEST AVAILABILITY

At the time our studies were initiated, *I. purchasi* was abundant in the field, and we assumed sufficient quantities could continuously be collected to feed to our *R. cardinalis* colony and run experiments. However, midway through the experiments, *I. purchasi* density declined because of drought, causing some experiments to be postponed. Additionally, some experiments were terminated early because some of the cottony cushion scales collected in the field were contaminated with mites or fungus. As a result, our colony had to be reduced in size to remove contaminants.

In retrospect, it would have been worth the investment of setting up a colony of *I. purchasi*. Although time consuming, this would have allowed us to have a continuous, uniform supply of the target pest. Maintaining the colony of *I. purchasi* under semi-quarantine conditions (i.e., in large cages) would also have eliminated contaminants.

EVALUATION OF FEEDING RANGE TESTS

DID WE TEST A WIDE ENOUGH RANGE OF POTENTIAL NON-TARGET SPECIES?

By including introduced species and a variety of native and endemic species in our tests, we were able to test neonate and mature *R. cardinalis* larvae against a wide range of species and demonstrate that *R. cardinalis* larvae have a narrow prey range.

Neonate larvae were tested against 35% (n = 17) of the homopteran species present in the Galápagos that were classified as potential non-target prey of conservation value. Mature larvae were tested against 29% of these species. Using endemic, native, and introduced species, we were able to test neonate and mature larvae against at least one species from each Homoptera family containing a species potentially at risk (Table 2). These test species included the endemic margarodid *M. similis*, which is the closest relative to *R. cardinalis*' usual prey (*I. purchasi*). Tests also included up to four species of above-ground mealybugs, the prey group most likely to be encountered by *R. cardinalis*, the group with the largest number of Galápagos endemics, and our highest priority for testing.

The smaller number of species tested with adult predators made reaching conclusions about adult prey range more difficult. Conditioned adults were tested against 29% (n = 17) of the high-risk Coccoidea, including representatives of four of the six families containing potential non-target prey. Naïve adults were tested on 23% of the high risk species and three of six families of interest. Testing of a wider range of species and repeating some trials would have been preferable, but extended dry periods following an El Niño event prevented this. We were unable to test adults on Ortheziidae, one of the closest families to the target prey. Trials with aphids were rendered invalid because of parasitization. Aphids, however, are distantly related and are unlikely to be used even as a temporary food source.

Definitive conclusions could not be reached about the extent of feeding of *M. similis*. Because only adults were tested, the possibility exists that eggs and early instars of *M. similis* might support *R. cardinalis* development; *R. cardinalis* has been shown to complete development on eggs but not adults of other genera of margarodids (Balachowsky, 1932; Mendel *et al*., 1998). Additional studies were not considered necessary because this species is subterranean and should not be exposed to the predator.

Unfavorable collecting conditions in the field also prevented us from sufficiently evaluating the interactions of Galápagos predators with *R. cardinalis*. Because this group of non-target species was under-represented in tests, we are unable to reach any conclusions about the potential interactions between the natural enemies of scale insects and *R. cardinalis*.

Thoroughness of Methods and Recommendations for Other Practitioners

The methods employed in this study were considered to be sufficiently rigorous to answer our questions about the feeding range of *R. cardinalis*. In practice, however, the lack of baseline data on the Galápagos Homoptera made it difficult to identify all species that might be affected by the introduction of *R. cardinalis*, while a small budget limited the number of field surveys that we could carry out to collect test species. The completeness of the assessment was also limited by testing *R. cardinalis* from only one geographic area. Testing *R. cardinalis* from different geographical locations would have had the advantage of increasing the genetic variability of the test material and reducing the risks of unpredicted non-target impacts associated with introducing the beetle from a geographical area in the event that it was no longer available from the original source.

Our limited budget forced us to devise cost-effective methods for testing this predator. Initial investment in obtaining literature allowed us to understand the behavior and biology of *R. cardinalis*, which helped us to determine the most appropriate test methods to use. Testing alternative species as family-level representatives of those non-target species that could not be located allowed us to test a greater number of species and complete the trials more quickly. The rationale used here was that, as long as we could define the prey range of *R. cardinalis*, it did not matter if we could not find all the non-target species desired for testing. In retrospect, it seems clear that the order in which species in such a program are tested can also influence the number of trials that need to be carried out. By defining the feeding range of *R. cardinalis* first, one can better identify the species that might be affected (by niche overlap, intraguild predation, or competition) and thus limit the number of species that need to be tested.

Our use of field-collected specimens in this study allowed us to quickly and cheaply test a wide variety of species. Nevertheless, it would have been better to rear at least the high priority test species in the laboratory. Testing field-collected material was deemed acceptable because few Galápagos Homoptera seemed to have parasitoids or pathogens (except for aphids). Nevertheless, field parasitization of introduced species reduced the range of species tested. The use of this method in areas where parasitism is higher would not be practical. Furthermore, because we didn't rear test species on their host plants, tests could not be carried out under even semi-natural conditions (as recommended by Sands and Van Driesche, 2003). This is, however, less important for coccinellids, which appear to respond to short-range cues associated with the prey (Dixon, 2000). Furthermore, our preliminary research and the findings of other authors, showed that the size of the test arena used in our experiments was unlikely to have influenced the feeding behavior of larvae or adults of *R. cardinalis*. For new projects, we recommend that reseachers compare the behavior of the predator in different test arenas before experiments are initiated. Finally, extensive efforts should be made to minimize effects of prey or host plant volatiles or plant structural defenses.

CONCLUSIONS

We summarize our findings in terms of the questions asked by the authorities and entomologists responsible for evaluating the proposed introduction of *R. cardinalis* to the Galápagos.

- **Could *R. cardinalis* survive in the long-term on Galápagos insects?** Out of a wide range of scale insects, neonates of R. cardinalis survived only on *I. purchasi* which suggests that the predator would be unable to complete its lifecycle and survive in the long term solely on other species from the Galápagos.

- **Are Galápagos insects suitable for *R. cardinalis* reproduction?** Test results with a small range of species indicated that, in the Galápagos, *I. purchasi* is the only species that is adequate for oogenesis of *R. cardinalis*. Additional tests are necessary to confirm this.

- **Could *R. cardinalis* adults and larvae survive temporarily on Galápagos insects in times of prey scarcity?** The only test species that supported any short-term feeding was the endemic species *M. similis*, the only other Margarodidae in the Galápagos. However, field studies have since shown that the subterranean habitat of this species makes it an improbable alternate prey for *R. cardinalis* (Causton *et al*., 2004). Test results suggest that neither young nor old larvae would be able to use above-ground Coccoidea species in the Galápagos as alternate prey. The prey range of adult *R. cardinalis* also appears to be narrow. However, additional trials are required to determine whether they are restricted to feeding on Margarodidae.

- **Does prior feeding experience influence prey selection?** Recently emerged larvae and adult *R. cardinalis* behaved the same as larvae and adults that had fed previously on *I. purchasi*, suggesting that prey selection was not influenced by prior experience with target prey.

- **Are damaging interactions likely with natives predators of scale insects?** Insufficient information due to a scarcity of necessary test insects prevents us from thoroughly evaluating the potential impact of *R. cardinalis* on native predators of Galápagos scales. However, intraguild predation and competition by *R. cardinalis* are doubtful because (1) *R. cardinalis* feeds specifically on Margarodidae and the only native predator of cottony cushion scale (the lacewing *C. cincta*) attacks larvae and weakened adults of *R. cardinalis* in captivity. Indeed, coccinellid larvae in general are susceptible to predation by lacewing larvae (Balduf, 1935; Bartlett, 1978; Sengonca and Frings, 1985; Waterhouse, 1991); (2) resident coccinellids and most other scale insect predators in the Galápagos do not feed on Margarodidae; (3) there is little habitat overlap between the prey of native coccinellids and *R. cardinalis*; and (4) *R. cardinalis* did not attack four commonly encountered species tested in these laboratory trials.

- **Is *R. cardinalis* safe to introduce into the Galápagos?** Results from our feeding range studies and risk assessment confirm the stenophagicity of *R. cardinalis* as previously reported (e.g., Quezada and Debach, 1973; Mendel and Blumberg, 1991; V. Brancatini, pers. comm., 1999).

The technical advisory committee of the CDF and the GNPS concluded that the potential detrimental effects of *R. cardinalis* on the environment and non-target organisms were minimal in relation to the immediate threat of endangered flora going extinct from damage by *I. purchasi*. Approval for *R. cardinalis*' release was granted in 2001, and over 1500 adult *R. cardinalis* have been liberated in priority areas on eight islands. Information is being gathered on the feeding behavior of the beetle in order to evaluate the effectiveness of *R. cardinalis* in reducing the target prey and its interactions with various Galápagos species.

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